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Forum

Improving Inferences from Hydrological Isotope Techniques

Camilla Ruø Rasmussen[1,2,*] and Andrew Kulmatiski[3]

Plant water isotopic compositions are widely used to describe patterns of soils water uptake. Although valuable, the technique only provides relative uptake distributions, which can be misleading. Without information on total transpiration, the technique cannot address central questions on drought response, competition, and species coexistence.

Water Uptake Distributions – A Valuable Piece in the Ecological Puzzle

Plant water uptake is among the most fundamental processes of terrestrial life. It is thought to play a major role not only in plant productivity but also in plant coexistence due to **niche partitioning** (see Glossary). There is increasing interest in understanding plant water uptake as climate change alters precipitation and evaporation patterns.

Plant water uptake is determined by several factors. Roots must be present and active in a soil depth to absorb water. Large, **suberized roots** transport but do not absorb soil water. For this reason, root biomass measurements often overestimate shallow root activity. Other measures of root presence, such as **root length density**, can also misrepresent water uptake because root activity varies due to differences in maturity and **aquaporin** abundance [1]. Furthermore, for water to flow into plant roots, the water potential of the soil must be greater than the water potential in plant roots. Finally, the amount of water absorbed by a plant is determined by the resistance to flow through the plant roots and stems and into the atmosphere. Plants with more leaf area or with larger stem or stomatal conductance will transpire more.

Hydrological isotope techniques have gained attention for their ability to describe vertical root activity distributions [2]. There are two broad classes of isotope techniques. Natural abundance techniques rely on natural variations in the composition of water **isotopologues** that can develop as a result of evaporative enrichment or stem from water sources such as rainwater or ground or surface water bearing different isotopic signatures. Artificial tracer techniques introduce water isotopologues into the soil. Both approaches use water isotope concentrations in stem and soil water in **mixing models** to produce an integrated measure of water uptake across the root system [3] and rely on the assumption that plants do not distinguish (i.e., fractionate) light from heavy water isotopologues, so the isotopic composition in the plant stem reflects the isotopic composition of the net water uptake.

A critical limitation of the techniques is that stem water isotopic composition does not provide a unique pattern of plant water uptake. Different distributions of water uptake can produce identical descriptions of plant stem water enrichment. Furthermore, a change in water uptake from one depth is confounded with changes in water uptake from other depths, preventing a localization of a plant's response to changes in water availability due to drought or competition. This limits the inferences on water uptake distributions, drought tolerance, and species coexistence, which can be drawn using hydrological isotope tracers. Introducing information on total transpiration to tracer studies can provide the missing link and allow researchers to

Glossary

Aquaporin: channel proteins that form pores in the membrane of biological cells, mainly facilitating transport of water between cells.
Isotopologues: molecules that differ only in their isotopic composition.
Mixing models: models used to quantify source contributions to a mixture.
Niche partitioning: the process by which natural selection drives competing species into different patterns of resource use, which is necessary for long-term coexistence.
Root length density: total length of roots per unit volume of soil.
Suberized roots: roots with a well-developed bark.

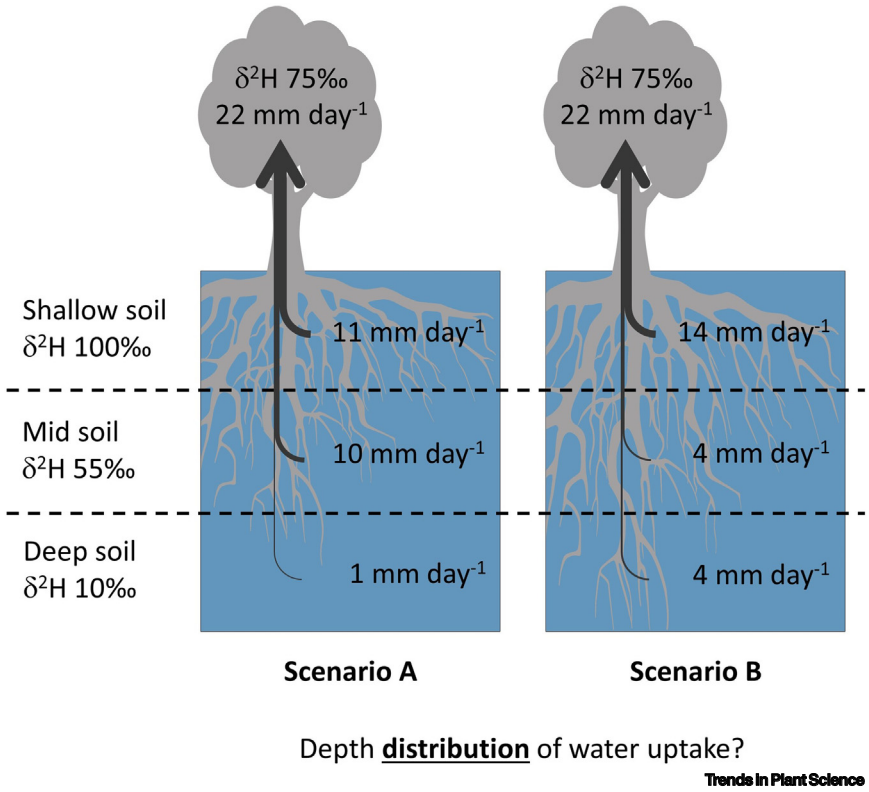

Figure 1. Ambiguous Uptake Distributions. Feeding water with specific isotopic compositions in plant stems and different soil depths into mixing models yields the relative contribution of water uptake from the various depths. When relying on the natural variation in the composition of water isotopes, there may be several solutions reflecting a specific stem isotopic composition. If, for example, 11 mm water day^{-1} is supplied from a shallow soil with an enrichment of δ^2H 100‰, 10 mm water day^{-1} is supplied from a midsoil with an enrichment of δ^2H 55‰, and 1 mm water day^{-1} is supplied from a deep soil with an enrichment of δ^2H 10‰, then the stem water enrichment will be 75‰ (scenario A). If the uptake from the three soil layers is 14, 4, and 4 mm day^{-1}, respectively, then the stem water enrichment will also be 75‰ (scenario B).

answer central questions on plant water use.

The Hidden Uptake Distribution

Natural abundance tracer studies frequently rely on the decreasing isotopic enrichment with depth, which is created because lighter isotopologues evaporate more readily than heavier ones, and diffusion of isotopologues distributes this effect downward. In regions with high evaporation rates, this profile extends to a depth of 20–50 cm [4]. Enrichment profiles caused by evaporation can be used to distinguish shallow from deep soil water use: plants containing stem water with heavier isotope ratios rely more on shallow than on deep soil waters.

A central problem for the natural abundance approach is that it is difficult to provide more than qualitative descriptions of vertical root water uptake patterns. For example, it is not possible to distinguish a plant that is using mostly top and mid-depth water (Figure 1, scenario A) from a plant that is using less mid-depth water but large amounts of deep water (Figure 1, scenario B).

Surviving or Thriving?

Hydrological isotope techniques are frequently used to determine whether plants increase deep water uptake in response to drought. Answering this question has important implications for plant productivity. However, two plants having the same ratio of deep to shallow water uptake can demonstrate the same stem isotopic composition even if one plant transpires more water than the other. This means that it is not possible to determine whether a drought only decreases shallow water uptake (Figure 2, drought scenario A) or whether the decreased shallow water uptake is to some extent compensated by an increase in deep water uptake (Figure 2, drought scenario B). Thus, it is not possible to know whether plants are surviving a drought or whether they can continue to grow on deep stored soil moisture [5]. This is essential in agricultural studies, where crop survival is not sufficient to secure high yields – the crop needs to thrive.

Therefore, reporting only the change in relative uptake depth evoked by drought [6–8] does not permit a distinction between these two scenarios. Because no inference can be made about whether water uptake deeper in the soil profile has increased, no information about how well plants cope with drought is provided.

Competing or Complementing?

Hydrological isotope techniques are also used to distinguish fundamental from realized hydrological niches of competing species [9]. Differences in vertical distribution of water uptake among species is assumed to play a role in species coexistence in water-limited environments. The method can distinguish fundamental from realized niches, but without information on total water uptake, stem isotopic composition cannot reveal the nature of the interaction among species.

Information on the relative water uptake distribution is not sufficient to determine whether a realized niche differentiation is a result of resource competition alone (Figure 2, coexistence scenario A) or whether resource complementarity also plays a role. It is the latter that can

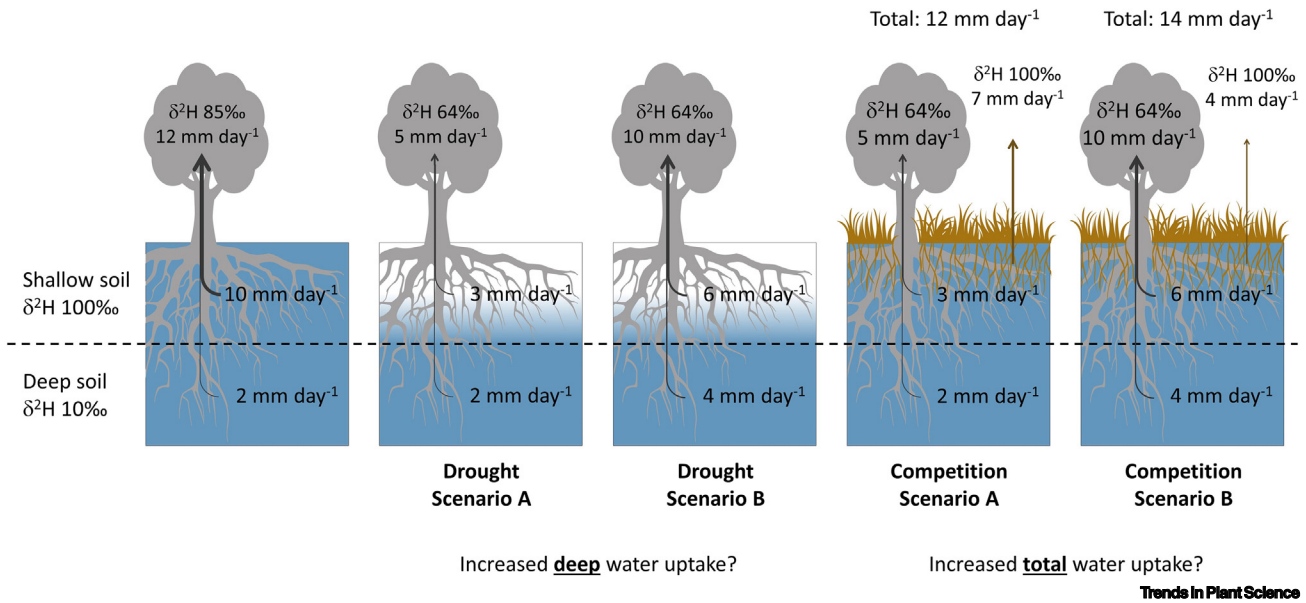

Figure 2. Ambiguous Outcome of Drought and Competition. The relative contribution of plant water uptake from distinct depths can be determined when the depths in question have distinct isotopic compositions, but the outcome of drought or intercropping cannot be revealed on the basis of relative contributions alone. If, for example, 10 mm water day^{-1} is supplied from a shallow soil with an enrichment of δ^2H 100‰ and 2 mm water day^{-1} is supplied from a deep soil with an enrichment of δ^2H 10‰, then the stem water enrichment will be δ^2H 85‰. If the shallow soil dries out or competition from another species decreases shallow water uptake to 3 mm day^{-1} but deep water uptake remains unchanged, then the drought or competition will decrease stem water enrichment to δ^2H 64‰ (drought scenario A and coexistence scenario A). If drought or competition only reduces shallow water uptake to 6 mm day^{-1}, however, but also results in an increase in deep water uptake to 4 mm day^{-1}, then the resulting stem water enrichment will still be 64‰ (drought scenario B and coexistence scenario B), leaving scenario A indistinguishable from scenario B, even though they can have substantially different outcomes for the plants. If reduced shallow water uptake during drought is to some extent compensated by deep water uptake, the plant is thriving better during the drought. If shallow soil competition leads to an increase in total water uptake in the system, coexistence increases the overall resource use in the system, leading resource complementarity.

be important for coexistence. Complementarity will only occur if interspecific interactions result in an increase in total resource use (Figure 2, coexistence scenario B). If interspecific interactions simply reduce deep-rooted species' access to shallow water without to some extent increasing deep water uptake, the effect is better described as competition than as complementarity. Observations reporting reduced biomass [10] are evidence of this alternative hypothesis, whereas overyielding in mixtures compared with monocultures hints at complementarity [7].

A Path Forward

We have identified two fundamental limitations of the use of stem water isotope approaches for understanding root dynamics. First, stem water isotopic compositions do not provide unique solutions to root water uptake distribution when based on a naturally occurring decreasing isotopic enrichment with depth. Second, stem water isotopic compositions do not provide information about total plant water uptake and are therefore unable to locate changes in the water uptake pattern induced by drought or competition. We suggest that the first problem can be resolved by injecting artificial tracers at specific soil depths. The second problem could be resolved by injecting artificial tracers in a quantitative budget approach similar to that used for nutrient isotope tracers (i.e., ^{15}N). Quantitative approaches are difficult to employ in water tracer studies because water flows and mixes quickly [11]. A simpler approach is to combine the tracer studies with quantitative measures or reliable estimates of transpiration [12].

In conclusion, anchoring relative water uptake measurements with measurements or estimates of absolute transpiration is necessary if we are to make strong inferences about plants' use of water from different soil depths.

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